*THE TRANSFER OF LEARNED BEHAVIOR FROM TRAINED TO UNTRAINED RATS BY MEANS OF BRAIN EXTRACTS, II**

By Frank Rosenblatt, John T. Farrow, and Sam Rhine

SECTION OF NEUROBIOLOGY AND BEHAVIOR, DIVISION OF BIOLOGICAL SCIENCES, CORNELL UNIVERSITY

Communicated by F. A. Long, December 20, 1965

In our previous paper[1] we presented experimental methods and results based on the pooled data of ten experiments on the transfer of learned behavior in rats. This paper contains a more detailed analysis of results of individual experiments, types of extracts, and dosage effects. As previously explained, raw data have been converted to standard scores, by taking the difference between observed and predicted scores for each rat ($O - P$) and dividing by the standard deviation of the corresponding control group. Since it was found that the top 50 per cent of the experimental rats and the top 50 per cent of the controls differed much more significantly than did the bottom 50 per cent of each group, the tables which follow present levels of significance for both the complete sample and for the top 50 per cent. Probabilities, in all cases, are obtained by a Mann-Whitney U test,[2] and numbers of experiments refer to the *Methods* section of our previous paper.

Table 1 summarizes results for the various tasks investigated. All tasks showed a significant tendency to transfer, with the possible exception of the discrimination of levers at the fixed light and dark ends of the Skinner box (expt. 8), which showed only a marginal effect. (The sample, consisting of only eight experimental and eight control rats, was insufficient to demonstrate statistical significance.) The alternating bar version of this experiment (item 6 in the table) showed a significant effect for the first test session, but with rapid extinction thereafter. Unfortunately, no control data were available for evaluating the significance of the activity level in this experiment, as distinct from the discrimination scores; from the raw data, it seems likely that the tendency to push the bar was in fact maintained at a high level, but that the discrimination between the light and dark ends of the box (which otherwise are identical to one another) was rapidly lost. In general, we have the impression that tasks which make use of a definite cue stimulus, such as a click, a buzzer, or a changing light, transfer more readily than operant conditioning tasks, particularly if the latter require an appreciable exertion of energy (such as pushing a bar, rather than merely approaching it). In order to obtain clear-cut results it also seems quite essential to reduce the "noise level" of the control animals by adequate preconditioning. The shorter preconditioning series used in our early experiments may partially account for our failure to show as clear a difference between experimental and control rats as was obtained by Babich *et al.* (see refs. 9–11 in paper I).

Activity measures (such as bar pressing) and discrimination measures (such as choice of bar in preference to nut, and choice of light or dark side) both show significant effects. The bar/nut discrimination is enhanced by adequate preadaptation to the indifferent stimulus (Table 1, 2nd and 3rd lines of item 4). When preconditioning was done without the nut, its sudden appearance in the test situation actually seems to produce a contrary bias in the low-dosage experimental groups (see Table 4, items 6, 7 and 10) possibly due to the transfer of an adaptation

TABLE 1

SUMMARY OF RESULTS FOR DIFFERENT TYPES OF TRAINING*

Training of donors	Extracts tested	N Exptl.	N Cont.	Performance measure	Mean standard score	Prob All rats	Prob Top 50%
1. Approach foodbox on click	RNA	60	20	Resp within 5″, 5 tests	0.446[1]	0.067	0.010
				Resp within 2″, 7 tests	0.908[1]	0.039	0.004
2. Cross on buzzer (shock)	Saline + phenol phase + interface	14	14	Resp within 4″, 6 tests	0.854[2]	0.025	0.006
3. Stay on safe side (dark for Exptl., light for Cont.)	" " "	8	8	Time on safe side, 6 tests	0.947[1]	0.065	0.014
4. Push bar for food	RNA, sol. 1000–10,000, and particle extracts; dose ≥ 1:1	92	82	Response rate	0.874[3]	0.005	0.00004
		32	62	Bar/nut comparison	0.510[1, 3]	0.021	0.006
		16	30	Same, with adaptation	0.840[3]	0.003	0.001
5. Push proper bar (dark for Exptl., light for Cont.)	Saline + phenol phases	8	8	% Resp on dark side, tests 1–2	0.386[1]	0.178	0.171
6. Change bars on light signal (Dark for Exptl., light for Cont.)	" " "	8	8	% Correct, test 1	1.368[1]	0.041	0.029
				tests 1–2	0.646	0.206	0.057
				tests 1–4	−0.054	0.520	0.707

* Standard score = $(O - P)/\sigma$, where O = observed score, P = predicted score, and σ = standard deviation of $(O - P)$ for corresponding control group. Derivations of predicted scores are indicated by superscripts: [1] Predicted score = mean score of control group; [2] Predicted score predicted from simultaneous activity, by control group regression line; [3] Predicted score predicted from preinjection score by control group regression line. Prob = probability that null hypothesis is correct, computed by single-tailed Mann-Whitney U test. N = no. of rats.

TABLE 2

EFFECTS OF TRAINING TIME AND DOSAGE*

Conditions	Bar-Pressing Rate N Exptl.	N Cont.	Mean standard score	Prob All rats	Prob Top 50%	% Bar vs. % Nut Responses N Exptl.	N Cont.	Mean standard score	Prob All rats	Prob Top 50%
(*a*) Training time (days):										
0	32	—	0.087	—	—	15	—	0.199	—	—
5	31	32	−0.093	0.788	0.714	15	15	−0.199	0.829	0.861
10	32	63	0.708	0.033	0.008	16	30	0.631	0.027	0.014
15	32	63	0.557	0.187	0.005	16	30	0.068	0.417	0.475
(*b*) Dosage, in brains per rat:										
1.4	32	32	0.663	0.045	0.003	16	30	0.840	0.003	0.001
1.0	70	60	0.766	0.079	0.007	—	—	—	—	—
0.7	32	31	0.601	0.250	0.035	16	30	−0.142	0.727	0.922

* From expts. 5 and 6; combined data from soluble and particulate fractions. Dosage of 1.0 comes from other bar-pressing experiments. Standard scores and pro babilities derived as in Table 1.

effect for the bar which is absent for the nut. With a high dosage, however, and with both manipulanda available during preconditioning, the bar/nut discrimination gives a clearly significant effect in the proper direction.

Factors which seemed likely to influence the potency of the effect, for a given extract, are considered in Tables 2 and 3. The effect of training time, based on data from experiments 5 and 6, suggests a possible peak in transferability at the time that the task has just reached its asymptotic value, but has not yet become fully automatic (about 10 days, in these experiments). Both the bar-pressing rate and the bar/nut discrimination scores show maxima for the 10-day group. Dosage is seen to have a quite critical effect, with only marginal effects being found when it is reduced to less than 1 brain per rat, and quite striking effects being evident with dosages greater than 1 brain per rat. The one case which was inconsistent with this conclusion was the particulate extract from experiment 6 (item 11 in Table 4), where a strong activity effect was found with the lower dosage, and a possible negative bias with the higher dosage. Since this fraction was later found to be responsible for several cases of phenol poisoning, however, it is likely that the negative activity measure (which is contradicted by a clearly significant discrimination measure) is attributable to the harmful effect of the extract.

The comparison of individual (1:1) with pooled brain transfer, shown in Table 3, shows no consistent bias in either direction. Both methods give significant transfer, and no significant difference is found between them.

The various extraction procedures and chemical treatments are compared in Table 4. The most noteworthy results here are: (1) significant transfer is obtained from all portions of the brain tested, including both cerebral and cerebellar fractions. Note, however, that the cerebellar effect, even with a dosage of 2 brains per rat, is much weaker than the cerebral and whole brain fractions. (2) Incubation with RNase, in which the RNA was demonstrated to have been destroyed, does not eliminate the effect. (3) One of the most potent extracts found was the soluble fraction with molecular weight range from 1,000 to 5,000, precipitated with acetone. (In general, acetone precipitation seems to give better results than ethanol precipitation, although a controlled comparison has not been made between them.) A marginal effect seems to be present in the molecular weight fraction from 5,000 to 10,000, and none at all in molecular weights over 10,000. In all of these cases, effects found with large dosages tended to vanish with low dosages. These results seem consistent with Rosenblatt's prediction of a molecular weight of about 4,000. (4) Extracts from the particle fraction, and injections of the particle material

TABLE 3

INDIVIDUAL VERSUS POOLED EXTRACTS

Training of donors	Type of extract	N		Mean stand. score	Prob	
		Exptl.	Cont.		All rats	Top 50%
1. Cross shuttle box on buzzer	Pooled	7	14	1.084	0.031	0.021
	Individ.	7	14	0.641	0.097	0.055
2. Stay on safe side of shuttle box	Pooled	4	4	0.970	0.171	—
	Individ.	4	4	0.914	0.100	—
3. Bar pressing	Pooled	10	10	0.411	0.273	0.155
	Individ.	10	10	0.793	0.075	0.016
4. Combined data	Pooled	21	28	0.742	0.031	0.0006
	Individ.	21	28	0.774	0.037	0.004
5. Individual (exptl.) vs. pooled (exptl.)	—	21	21	—	0.684	0.745

TABLE 4

SUMMARY OF RESULTS FOR DIFFERENT TYPES OF EXTRACTS*

Extract	Training	Dosage	N		Activity Scores			Discrimination Scores		
			Exptl.	Cont.	Mean stand. score	Prob All rats	Prob Top 50%	Mean stand. score	Prob All rats	Prob Top 50%
1. RNA (saline phase)	1, 4	1.0	90	40	0.445	0.058	0.003	—	—	—
Cerebral	1	1.0	20	20	1.197	0.088	0.029	—	—	—
Cerebellar	1	2.0	10	10	0.288	0.012	0.022	—	—	—
Whole brain	1	1.0	10	10	1.468	0.180	0.028	—	—	—
2. RNA + RNase	1	1.0	20	20	0.549	0.033	0.019	—	—	—
3. Saline + phenol phase	5, 6	1.0	16	16	—	—	—	0.516	0.107	0.019
4. Saline + phenol + interface	2	1.0	14	14	0.854	0.025	0.006	—	—	—
	3	1.0	8	8	—	—	—	0.947	0.065	0.014
5. Extract 4 + residual ppt after 3 days	2	1.0	13	14	1.145	0.030	0.019	—	—	—
	3	1.0	8	8	—	—	—	0.840	0.107‡	0.014
6. Soluble 1,000–5,000	4	1.4	8	8	1.859	0.002	0.014	0.393†	0.325‡	0.500
		1.0	10	10	1.508	0.065	0.048	—	—	—
		0.7	8	8	−0.065	0.480	0.657	0.054†	0.824‡	0.935
		1.0, 1.4	18	18	1.659	0.006	0.0015	—	—	—
7. Soluble 5,000–10,000 (plus heavier weights in some extracts)	4	1.4	8	8	1.155	0.080	0.057	0.825	0.016‡	0.023
		1.0	10	10	0.305	0.145	0.274	—	—	—
		0.7	8	7	0.000	0.653	0.343	−0.386	0.903‡	0.945
		1.0, 1.4	18	18	0.683	0.046	0.061	—	—	—
8. Soluble > 10,000	4	1.0	10	10	−0.563	0.943	0.952	—	—	—
9. Particle extract, saline phase	4	1.0	10	10	2.560	0.184	0.004	—	—	—
10. Particle extract, saline + phenol phases	4	1.4	8	8	0.215	0.191	0.243	−0.090†	0.343‡	0.935
		0.7	8	8	0.118	0.439	0.443	−0.085†	0.963‡	0.773
11. Residual ppt from 10	4	1.4	8	8	−0.556	0.809	0.757	0.855	0.017‡	0.003
		0.7	8	8	2.352	0.041	0.029	0.103	0.359‡	0.726
12. Extract 11 + trypsin	5, 6	0.5–1.0	16	15	—	—	—	−0.134	0.460	0.041
Soluble phase		0.5–1.0	8	7	—	—	—	−0.540	0.347	0.057
Residual ppt		1.0	8	8	—	—	—	0.272	0.460	0.171
13. Extract 11 + chymotrypsin	5, 6	0.5–1.0	16	16	—	—	—	0.403	0.203	0.253
Soluble phase		0.5–1.0	8	8	—	—	—	0.127	0.561	0.829
Residual ppt		1.0	8	8	—	—	—	0.680	0.080	0.014
14. Saline soln. vs. RNA control	4	1.0	10	10	0.411	0.273	0.210	—	—	—

* Numbers in "Training" column refer to entries in Table 1. Dosage in donor brains per recipient. Standard scores and probabilities computed as in Table 1.
† Rats preconditioned with bar only; nut introduced during test sessions.
‡ The control group used for computing these probabilities included all controls from a given experiment, regardless of dosage or type of extract (N = 30 to 32).

TABLE 5

SUMMARY OF EVIDENCE ON NATURE OF MOLECULE

Observations	References*
Evidence for RNA:	
1. Transfer obtained from brain extracts containing RNA	1, 2, 3
Evidence against RNA:	
1. Transfer obtained from intraperitoneal injection	1, 3, 4
2. Activity persists after RNase incubation	3, 4
3. Molecular weight under 5,000	3
4. Transfer eliminated by chymotrypsin	3(?), 4
5. Soluble in phenol	3, 4
6. Insoluble in acetone	3, 4
Evidence for polypeptide:	
1. Folin-Ciocalteu test shows peptide present	3
2. Soluble in phenol, insoluble in acetone and ethanol	3, 4
3. Molecular weight between 1,000 and 5,000	3
4. Transfer eliminated by chymotrypsin	3(?), 4
Evidence against polypeptide:	
1. Biuret test negative for protein	1

* References: (1) Babich *et al.*, (2) Fjerdingstad *et al.*, (3) Rosenblatt *et al.*, and (4) Ungar *et al.*, as previously cited by Rosenblatt *et al.*, these PROCEEDINGS, **55**, 554 (1966).

itself, seem to give results at least as strong, or stronger, than those obtained from the soluble fractions. The most potent extracts of all, as measured by the mean standard score for activity measurements, were the particle extract from experiment 4 (item 9) and the residual precipitate from experiments 5 and 6 (item 11), despite the problems already noted for this last extract. These results seem to be consistent with the notion of an adhesive molecule, binding to pre- and postsynaptic membranes. (5) The enzyme treatments give results which seem consistent with the hypothesis that trypsin does not attack the information-carrying molecule, but does attack its binding sites, releasing it from the particulate fraction into solution, while chymotrypsin leaves the bound form of the information molecule (and its binding site) intact, but may destroy the molecule in its soluble form. This interpretation would be consistent with the finding of Ungar and Oceguera-Navarro that their effect was immune to trypsin but destroyed by chymotrypsin (see ref. 12 in paper I). (6) The comparison of saline injections with control RNA extract suggests a possible depressing effect of the brain extract, although this is not statistically significant. By contrast, the brain extracts in experiment 7 appeared to have a transient activating effect on the controls as well as the experimental group, from both the initial injection and the "booster shots." It is also worth noting that the effects of negative reinforcement, in this case, seemed to be still manifest after 6 days (12 sessions) of extinction trials (item 5 of Table 4).

Discussion.—Two main issues which have seemed uncertain in terms of some of the earlier work on "memory transfer" seem to be clarified by our findings: (1) A wide variety of tasks, including classical conditioning, operant conditioning, and discrimination tasks, with both positive and negative reinforcement, seem to be susceptible to transfer, and (regardless of any additional generalized activating influence which may be present) the transfer is specific to the learned task. (2) The information-bearing molecule seems to have properties more consistent with those of a polypeptide, adhering to the cell membrane, than with RNA. The evidence for and against the RNA and polypeptide alternatives is summarized in Table 5, which includes both our own findings and those of other investigators, where these bear directly on the phenomenon in question. Indirect evidence (such as Hydén's

finding of altered base ratios after learning, and evidence from invertebrates) is not included here. While it is still conceivable that a hybrid molecule, consisting partly of a "protected" form of polynucleotide, might correspond to the discovered list of properties, this seems to be an unparsimonious assumption to make without further evidence, and it also seems questionable whether such a molecule could contain the required quantity of information in the small number of nucleotide groups possible within the molecular weight constraints. In any event, further work on the properties and subcellular localization of the molecule seems to be required in order to obtain a definitive answer to this question, and to permit a clear choice between the alternative theories of synaptic modification and genetic induction as the underlying mechanism for the transfer effect.

* This work was supported by U.S. Office of Naval Research contract Nonr 401(40) and National Science Foundation contract GK-250.

[1] Rosenblatt, F., J. T. Farrow, and S. Rhine, these PROCEEDINGS, **55**, 548 (1966).

[2] Siegel, S., *Nonparametric Statistics* (New York; McGraw-Hill, 1956).

---

*ISOLATION OF THE DNA OF THE E. COLI CHROMOSOME IN ONE PIECE**

BY C. I. DAVERN

COLD SPRING HARBOR LABORATORY OF QUANTITATIVE BIOLOGY, COLD SPRING HARBOR, NEW YORK

Communicated by A. D. Hershey, February 10, 1966

The autoradiographs of H^3-labeled chromosomes of *Escherichia coli* prepared by Cairns,[1] and the explanations of the chromosome's growing mechanism by Bonhoeffer and Gierer[2] and Nagata,[3] are consistent with the notion that the DNA of the replicating chromosome is a single molecule with a single growing point. Such a molecule would have a molecular weight of $2.3–4.6 \times 10^9$ daltons,[1] depending on its stage of replication. Attempts to isolate the DNA of the *E. coli* chromosome in one piece, by methods designed to minimize shear and nuclease action, have so far failed,[4, 5] although very large DNA molecules (4×10^8 daltons), corresponding roughly to half the nuclear DNA content, have been isolated from *Hemophilus influenzae* by Berns and Thomas.[5]

Such attempts to relate the isolated DNA molecule to the molecular organization of the bacterial chromosome are indirect, for they rely on comparison of an estimate of the molecular weight of the isolated molecule with an estimate of the chromosome DNA content. This paper describes a more direct method for assessing the relation between the isolated DNA molecule and the DNA of the chromosome. The method was applied to DNA extracted from *E. coli* and banded in a CsCl density gradient by means of a technique designed to avoid shear stress and minimize nuclease action. It showed that the DNA of the *E. coli* chromosome can be isolated in one piece.

The assessment is based on CsCl equilibrium density gradient analysis[6] of DNA isolated from cells that have incorporated 5-bromouracil into their DNA for a portion of the chromosome replication cycle. If each chromosome has all its DNA in